

Nocturnal, diurnal, crepuscular: activity assessments of Pisauridae and Lycosidae

Robert B. Suter: Department of Biology, Vassar College, 124 Raymond Avenue, Poughkeepsie, NY 12603, USA. E-mail: suter@vassar.edu

Kari Benson: School of Sciences, Lynchburg College, 1501 Lakeside Dr., Lynchburg, VA 24501, USA

Abstract. Animals are commonly categorized as diurnal, nocturnal, or crepuscular depending on the times of day when they are most active. These categories, although convenient, would be more useful if we knew more about how closely animal activity conformed to the labels. Similarly, if we knew more about the degree of nocturnality or diurnality of a particular species, we would have increased understanding of the selective forces acting on it. To clarify the intensity of nocturnality or diurnality in lycosoid spiders, we measured activity in 46 spiders divided among three congeneric species of fishing spiders (Pisauridae) and five species of wolf spiders (Lycosidae), in an austere laboratory setting. Overall, the three pisaurid species, pooled, were less than half as active as the five lycosid species, also pooled. All three species of fishing spiders and four of the five species of wolf spiders were strongly nocturnal in their activity. Only one species, the wolf spider *Piratula minuta* (Emerton 1885), was diurnal. None of the individual spiders that showed statistically significant nocturnal or diurnal activity (31 of 31 lycosids, 14 of 15 pisaurids) was purely nocturnal or diurnal. In all individual cases except for a single ambivalent *Dolomedes tenebrosus* Hentz 1844 (Pisauridae), statistically strong nocturnality was accompanied by substantial activity during the light hours, and statistically strong diurnality was accompanied by substantial activity during the dark hours. We discuss the overall low variability in activity patterns among the fishing spiders in comparison to the much higher variability among the activity patterns of the wolf spiders, the common but not ubiquitous presence of ultradian periodicities in individual spiders, and the significance of the fact that none of the individual spiders was strictly nocturnal or strictly diurnal.

Keywords: Circadian rhythm, activity monitor, syntopy, temporal partitioning, bouts

The terms nocturnal, diurnal, and crepuscular invite categorical assumptions about the habits of organisms. That categorical perspective does not reflect reality for some spiders (e.g., McQueen & Culik 1981; Schmitt et al. 1990; Suter 1993; Schmitz 2004). Even spiders with strong nocturnal or diurnal periodicity might exhibit movement during the part of the day when the spider is less active because it may still be susceptible during that “inactive” period to predatory attacks (Blackledge et al. 2003; Jones et al. 2011), parasitoid discovery (Finch 2005), burrow flooding (Rovner 1987), web destruction (Eisner & Nowicki 1983; Blackledge & Wenzel 1999), and other natural hazards, to say nothing of the fortuitous appearance of prey. On the other hand, spiders are known for having long periods of relative inactivity, correlated with lower than usual metabolic rates (Anderson 1970; Greenstone & Bennett 1980; Schmitz 2004), so perhaps nocturnal torpor is typical for some diurnal spiders, and diurnal torpor is typical for some nocturnal spiders. How rigidly spiders temporally partition their activity has ecological, behavioral, and physiological consequences.

Ecologically, a strictly diurnal spider species, exhibiting no activity during the night, could presumably coexist with a strictly nocturnal spider species even if the two species were otherwise identical, their lives syntopic but asynchronous (e.g., Herberstein & Elgar 1994; Kronfeld-Schor & Dayan 2003). On the other hand, more relaxed partitioning, accompanied by competition and perhaps predation, may be relatively common (e.g., Schmitt et al. 1990; see also discussion and references in Kronfeld-Schor & Dayan 2003). Periodicities in behavioral activity patterns, whether rigid or flexible, can incur additional costs. For example, in some nocturnal fishing spiders (Pisauridae), the presence of strong light during the

day causes or triggers degradation of the eyes' photoreceptors, necessitating their reconstruction at dusk (Blest & Day 1978), an oscillation that is also known in some Lycosidae where the changes are linked to the circadian rhythm (Kovoor et al. 1995, 1999). In addition, the physiological consequences of environmental thermal and humidity regimes, tightly coupled in some climates, may demand very different behavioral responses if the spider is to maintain physiological homeostasis (e.g., Humphreys 1974, 1975). Further, because both predator and prey suites can differ substantially, the economics of being primarily nocturnal but still active during daylight hours, or vice versa, could require substantial accommodation (e.g., Blackledge & Wenzel 1999; Nørgaard et al. 2006).

For the current study, we developed an IR motion detection system to monitor the activity of three species of fishing spiders (Pisauridae) and five species of wolf spiders (Lycosidae) for 48 h in a 12:12 light:dark laboratory setting. This system is quite sensitive to small motions and can thus support detailed analyses of temporal patterning in behavior. We tested 1) whether pisaurids and lycosids differ in overall activity, 2) whether, at both diel and finer scales, nocturnal pisaurids and lycosids partition their activity differently and 3) whether our species showed strictly diurnal or nocturnal activity patterns.

METHODS

Spiders.—We gathered activity data on three species of fishing spiders (Araneae: Pisauridae) and five species of wolf spiders (Araneae: Lycosidae). The pisaurids, *Dolomedes tenebrosus* Hentz 1844, *D. triton* (Walckenaer 1837), and *D. vittatus* Walckenaer 1837, were collected from sites in Bedford County, Virginia, and Dutchess County, New York, in

September and October 2012. The lycosids, *Gladicosa pulchra* (Keyserling 1877), *Hogna lenta* (cf. *Lycosa lenta* (Hentz 1844) sensu Wallace 1942), *Rabidosia punctulata* (Hentz 1844), and *Varacosa avara* (Keyserling, 1877) were collected in Lafayette County, Mississippi, in September and November 2012; *Piratula minuta* (Emerton 1885) were collected in November 2012 in Dutchess County, New York. In all taxa, our subjects included only mature or penultimate females.

Each captured spider was maintained in a 240-ml polystyrene cup topped by the inverted lower half of a plastic Petri dish (100 mm diameter) and provided with water via a fiber wick leading from a small reservoir below the cup. We did not feed the spiders during their 3–21-day maintenance period. During the entirety of their captivity, spiders were held in the same light-tight chamber (1.2 × 3.0 × 2.4 m) that housed the activity monitoring apparatus, and they therefore were under the same 12:12 LD lighting regime that we used during data collection. Under that regime, an electronic timer turned on two fluorescent lights at 0600 h and turned them off at 1800 h, approximating the unshifted natural light cycle at the autumnal equinox. The lights were located about 1 m above not only the spider maintenance cups but also the activity monitoring apparatus, providing 487–495 lux of white light at the level of the spiders.

Activity monitoring.—After 3–18 days of acclimation to the 12:12 LD lighting regime, we coaxed each spider from its maintenance cup into a vial and thence into a plastic tissue-culture flask, which served as the spider's test chamber. Each chamber was sealed with wicking material saturated with distilled H₂O (for drinking) and with a screw-on plastic cap (for security). We used two sizes of flasks (300 ml and 60 ml, with 95 cm² and 30 cm² of floor space, respectively), the larger ones for the large spiders (the three *Dolomedes* species and *Gladicosa pulchra*, *Hogna lenta*, and *Rabidosia punctulata*), and the smaller ones for the other lycosids (*Varacosa avara* and *Piratula minuta*). The two flask sizes were paired with proportionately sized arrays of infrared LEDs and IR sensors. We performed the transfers from maintenance cup to test chamber, as well as daily checks of the status of the spiders and the activity monitoring system, between 1145–1214 h, the middle of the spiders' objective day, so that our activities would not alter the light regime. Because each experimental trial began at noon (1200 in clock time, 0 h in trial time), the two dark periods were from 6–18 h and from 30–42 h in the 48-h trial. Each test chamber rested on an open-cell foam pad, thereby providing a degree of vibrational isolation, and each chamber was visually isolated from other occupied chambers.

The motion detectors (Fig. 1) consisted of four components: the test chambers and the IR LED and IR sensor arrays mentioned above, simple electronic circuitry that produced a voltage proportionate to the amount of IR light falling on the IR sensors, a four-channel analog-to-digital converter (Lab-Pro, Vernier Software and Technology), and software (Logger Pro, v. 3.8.2, Vernier Software and Technology) that controlled the A/D converter and the storage of the incoming data. The four channels accommodated three test chambers and one visible light sensor (Vernier Software and Technology) used to confirm light levels at the test chambers.

Each activity monitoring session lasted 48 h, divided into segments alternating between light and dark in the sequence L,

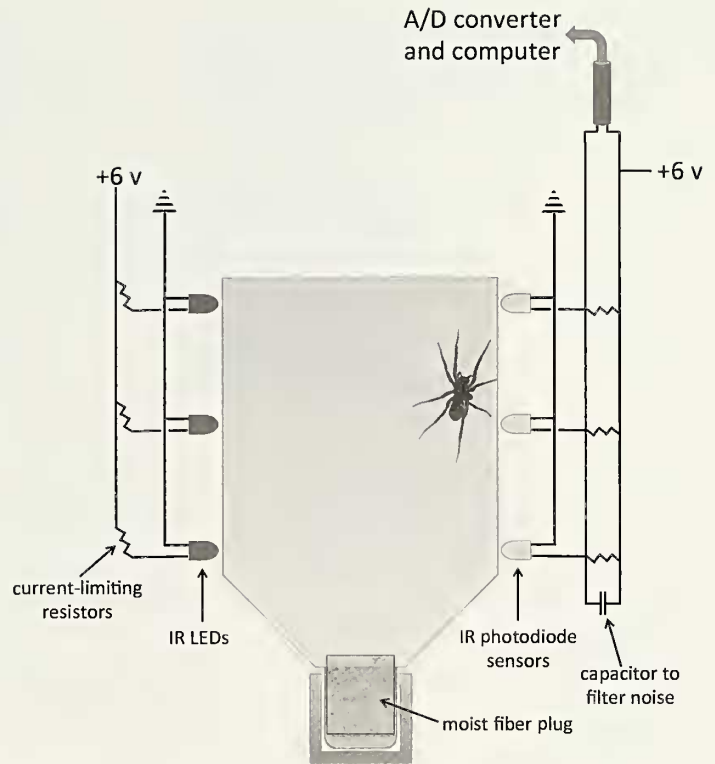


Figure 1.—Overhead view of an activity chamber (a tissue culture flask) and its associated electronics, shown approximately to scale. Because of the divergence of the IR light from each emitter, each IR sensor received light from at least two emitters.

6h; D, 12h; L, 12h; D, 12h; L, 6h. This provided us with two full 24-h cycles and four transitions between the two light levels.

We set the software to collect data at 15-s intervals from each test chamber and from the visible light sensor. The result, for each test chamber, was a string of 11,520 voltage measurements (4/min, 240/h, for 48 h).

Data analysis.—To determine whether activity had occurred during any 15-s interval, we subtracted the second datum from the first and took the absolute value of the difference as our raw measure of activity. This single measure was not quantitatively meaningful because, for example, a large change would occur if the spider had moved a long distance, perhaps starting at a position that fully occluded an IR sensor and ending at a position near the water supply (leaving all LED-to-sensor paths open); but a similarly large change would occur if the spider only shifted its position by 3–5 mm but its starting point just barely, but fully, occluded one of the IR sensors, and its ending point fully opened the IR light paths to that sensor. Thus the magnitudes of changes in voltages were not reliably related to proportional changes in activity. Therefore, we scored every interval as having movement (1) or no movement (0), without regard to the amount of movement suggested by voltage changes greater than zero, thereby giving equal weight to all instances of measurable activity.

The resulting series of 11,519 zeros and ones constituted our primary measure of activity, *binary activity*. To detect patterns of activity, some of which might not be readily discoverable by visual inspection of the data, we applied a discrete Fourier

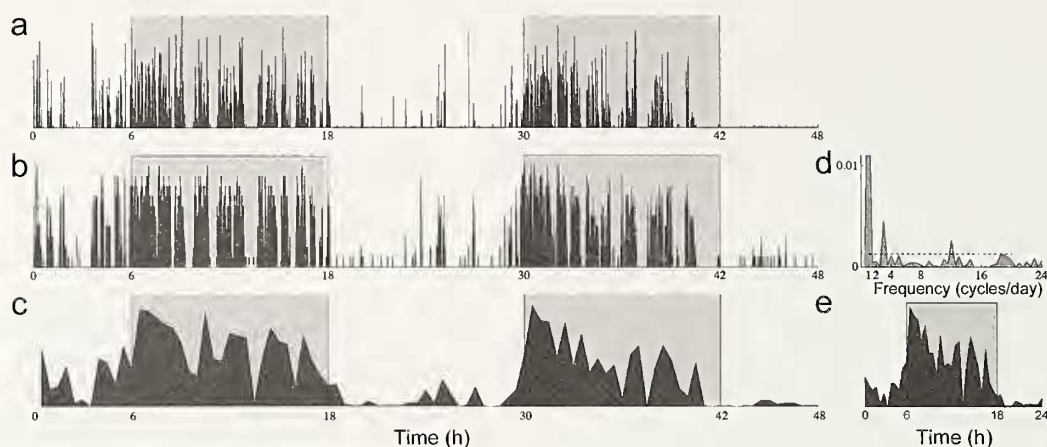


Figure 2.—Data treatments in the analysis of activity. a) Raw data (volts) from a 48-h trial with *V. avara*. Each vertical line shows the absolute value of the monitoring system's output at time t minus the output at time $t+15$ s. The shaded areas indicate when the lights were off. b) *Short-term activity* measured by first converting the raw data into 0s and 1s (forming *binary activity*), with 0 indicating no movement in a 15-s interval and 1 indicating movement, and then smoothing the data by taking a running average (10 intervals at a time, shifting by one interval at each iteration) of the string of 0s and 1s. This procedure gave an index of activity (range: 0.0–1.0 in steps of 0.1) for each 150 s of raw data. *Short-term activity*, in which higher values correspond to more activity, is comparable to the actographs typically used to display activity data in circadian rhythm studies. c) Activity envelope derived from the same string of 0s and 1s, averaged over 120 intervals (1800 s) for each value, and the 120-interval blocks were contiguous. d) Power spectrum generated by applying a discrete Fourier transform (DFT) to the string of 0s and 1s. Peaks above the horizontal dashed line are unlikely to have occurred by chance ($P < 0.01$), indicating that the 48 hours of activity revealed not only the expected circadian oscillation (1 cycle/day) but also oscillations at 3 and 12 cycles/day. e) The characteristic *activity profile* for this spider, formed by averaging the two 24-h blocks of data shown in (c).

transform to *binary activity* (Forrest & Suter 1994; Suter & Forrest 1994). The output from that procedure (Fig. 2d) is a power spectrum showing what proportion of the variance in the time series is attributable to underlying fluctuations at frequencies of one per time period, two per time period, and so forth. In our data, the relevant time period was 24 h, so the corresponding frequencies are expressed as 1/day, 2/day, etc. We adopted a conservative $\alpha = 0.01$ for the significance of peaks on the power spectra and only evaluated peaks in the

range 1–20 cycles/day. Our procedure followed Forrest & Suter (1994) and Suter & Forrest (1994).

We also used *binary activity*, the series of zeros and ones, to count the number of intervals during which a spider was active under light (L) and in darkness (D), and then to calculate the spider's $(L-D)/(L+D)$ ratio, a relative estimate of diurnality or nocturnality.

We constructed a view of the intensity of a spider's activity over short time periods by taking a running average of *binary*

Table 1.—The pisaurids were strongly nocturnal in their behavior, with a single ambivalent exception (shaded). ANOVA of the $(L-D)/(L+D)$ ratios of the pisaurids, including the ambivalent *D. tenebrosus* outlier, showed no significant differences among them ($F_{2,12} = 0.1499$, $P = 0.8624$). We compared these pooled pisaurids with the pooled nocturnal lycosids (Table 2; excluding the diurnal *P. minuta*) and found the lycosids to be more strongly nocturnal than these pisaurids (two-tailed $t_{38} = 2.728$, $P = 0.0096$).

	Light	Dark	$(L-D)/(L+D)$	χ^2	P
<i>D. triton</i>	372	448	-0.093	6.9	< 0.01
	96	344	-0.564	139.8	< 0.0001
	87	400	-0.643	201.2	< 0.0001
	372	953	-0.438	254.9	< 0.0001
	211	256	-0.096	4.3	< 0.05
	$(L-D)/(L+D)$ mean \pm SE		-0.367 \pm 0.116		
<i>D. vittatus</i>	250	456	-0.292	60.1	< 0.0001
	448	725	-0.236	65.5	< 0.0001
	273	449	-0.244	42.9	< 0.0001
	306	595	-0.321	92.5	< 0.0001
	168	516	-0.509	177.1	< 0.0001
	$(L-D)/(L+D)$ mean \pm SE		-0.320 \pm 0.050		
<i>D. tenebrosus</i>	171	344	-0.336	58.1	< 0.0001
	330	662	-0.335	111.2	< 0.0001
	347	391	-0.060	2.6	> 0.05
	246	821	-0.539	310.0	< 0.0001
	373	615	-0.245	59.3	< 0.0001
	$(L-D)/(L+D)$ mean \pm SE		-0.303 \pm 0.078		

Table 2.—The lycosids were strongly nocturnal in their behavior, with the exception of 1) a single *V. avara* (shaded) that was more diurnal than would be expected by chance and 2) *P. minuta*, all of which were strongly diurnal. ANOVA of the (L–D)/(L+D) ratios of the nocturnal lycosids, excluding the *V. avara* outlier, showed no significant differences among them ($F_{3,21} = 1.967$, $P = 0.1499$). We compared these pooled nocturnal lycosids with the pooled pisaurids (Table 1) and found the pisaurids to be more weakly nocturnal than these lycosids (two-tailed $t_{38} = 2.728$, $P = 0.0096$).

	Light	Dark	(L–D)/(L+D)	χ^2	<i>P</i>
<i>H. leuta</i>	210	1102	–0.680	606.6	< 0.0001
	294	650	–0.377	129.5	< 0.0001
	404	1136	–0.475	345.9	< 0.0001
	524	1973	–0.580	836.8	< 0.0001
	490	1465	–0.499	483.5	< 0.0001
	262	922	–0.557	367.4	< 0.0001
	(L–D)/(L+D) mean \pm SE		–0.528 \pm 0.042		
<i>G. pulchra</i>	517	2987	–0.705	1735.1	< 0.0001
	440	1055	–0.411	251.5	< 0.0001
	291	1004	–0.551	390.8	< 0.0001
	559	1980	–0.560	791.8	< 0.0001
	378	770	–0.341	131.2	< 0.0001
	287	877	–0.507	295.1	< 0.0001
	(L–D)/(L+D) mean \pm SE		–0.513 \pm 0.052		
<i>R. punctulata</i>	438	2095	–0.654	1084.2	< 0.0001
	665	2596	–0.592	1117.3	< 0.0001
	146	419	–0.483	131.2	< 0.0001
	130	269	–0.348	48.3	< 0.0001
	174	522	–0.500	173.5	< 0.0001
	128	284	–0.379	58.8	< 0.0001
	(L–D)/(L+D) mean \pm SE		–0.493 \pm 0.048		
<i>V. avara</i>	353	998	–0.477	306.4	< 0.0001
	412	808	–0.325	127.6	< 0.0001
	543	1576	–0.487	503.8	< 0.0001
	958	758	0.117	23.3	< 0.001
	2114	3648	–0.266	408.7	< 0.0001
	2392	2934	–0.102	55.1	< 0.0001
	922	1632	–0.278	197.3	< 0.0001
	422	1861	–0.630	906.8	< 0.0001
		(L–D)/(L+D) mean \pm SE		–0.367 \pm 0.067	
<i>P. minuta</i>	173	40	0.624	83.0	< 0.0001
	327	140	0.400	74.8	< 0.0001
	372	126	0.494	121.5	< 0.0001
	1031	498	0.349	185.7	< 0.0001
	503	258	0.322	87.8	< 0.0001
	(L–D)/(L+D) mean \pm SE		0.438 \pm 0.055		

activity, using 10 intervals for each average and shifting one interval for each iteration. This gave us our secondary measure of activity, *short-term activity*, composed of a series of indices of activity that ranged from 0–1 in steps of 0.1 (Fig. 2b), with each point being the mean of 150 s of *binary activity*. The raw data (Fig. 2a) and *short-term activity* (Fig. 2b) are visually quite similar, although *short-term activity* is more meaningful because its values reflect the degree to which activity, however energetic or lethargic, was sustained.

Our tertiary measure of activity constituted an activity envelope (Figs. 2c, e), derived from the same string of 0s and 1s that make up *binary activity*, but this time averaged over 120 intervals (1800 s) for each value, and the 120-interval blocks were contiguous (rather than overlapping as was the case with the running average that led to *short-term activity*). Again, the raw data (Fig. 2a) and *short-term activity* (Fig. 2b) and the activity envelope (Fig. 2c) are visually quite similar. For each individual spider, we averaged the two halves of the

activity envelope and refer to the resulting plot as the characteristic *activity profile* for that spider.

We used Mathematica 8.0 (Wolfram Research) to perform all of the data manipulations described above and in Fig. 2. Further analyses of the Mathematica outputs, including χ^2 , t-tests, ANOVA, and multiple regressions, were performed in Prism 4.0 (GraphPad Software).

RESULTS

Total activity.—The total activity scores of the three species of fishing spiders (Table 1, light + dark) did not differ significantly (ANOVA: $F_{2,12} = 0.424$, $P = 0.664$). The total activity scores of the five species of wolf spiders (Table 2) showed marginally significant variability (ANOVA: $F_{4,26} = 2.766$, $P = 0.048$), the only significant difference in pairwise tests being between the most active *V. avara* and the least active *P. minuta* (Tukey's Multiple Comparison Test: $q = 4.372$, $P < 0.05$).

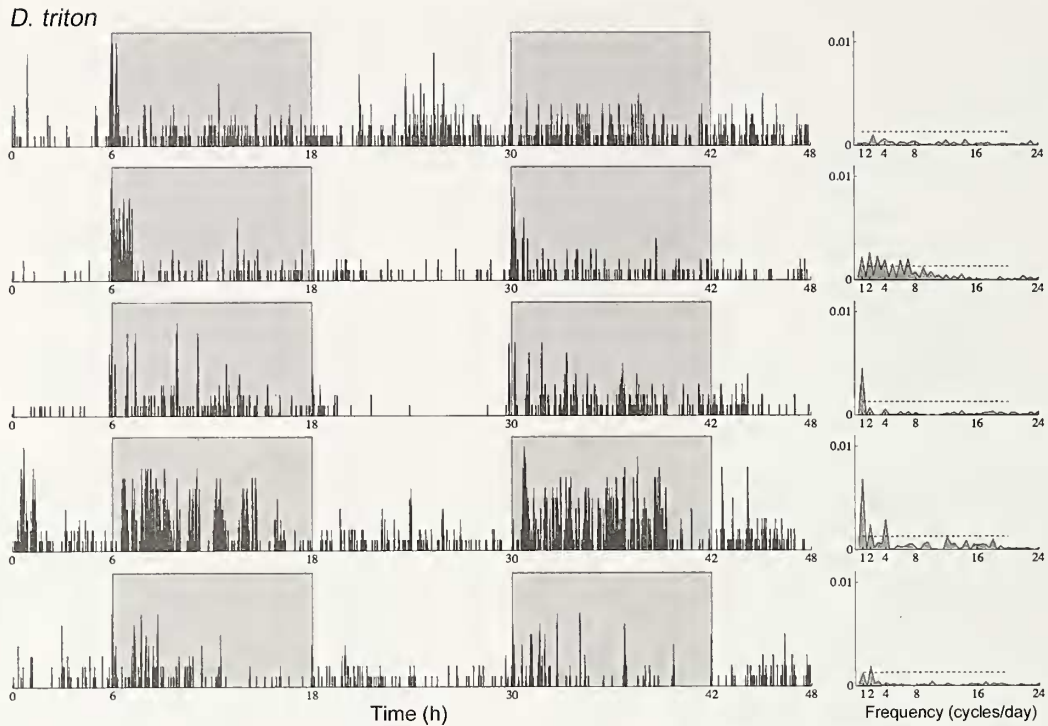


Figure 3.—*D. triton* (Pisauridae) *short-term* activity (left) and power spectra (right). All but the last of these spiders were demonstrably nocturnal in their activity; the last spider's activity was only marginally nocturnal, with the greater amount of its activity occurring in the dark but that had slightly less than a 5% likelihood of occurring by chance (Table 1). The power spectra show that the middle three spiders' activity patterns were periodic at about one cycle per day whereas the first and last spiders were not obviously circadian in their activity. The first spider's activity was approximately aperiodic as assessed with the discrete Fourier transform.

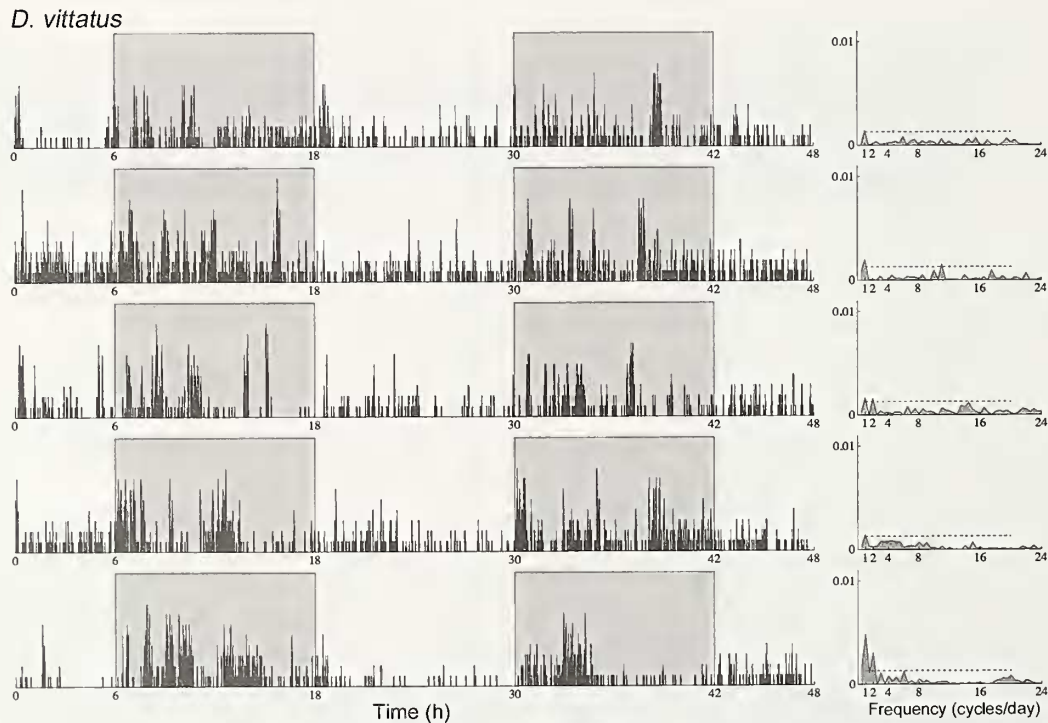


Figure 4.—*D. vittatus* (Pisauridae) *short-term* activity (left) and power spectra (right). All of these spiders were strongly nocturnal in their activity (Table 1), and all had activity patterns that were periodic at about one cycle per day as shown in the power spectra. Three of the spiders also had significant periodicities at higher frequencies.

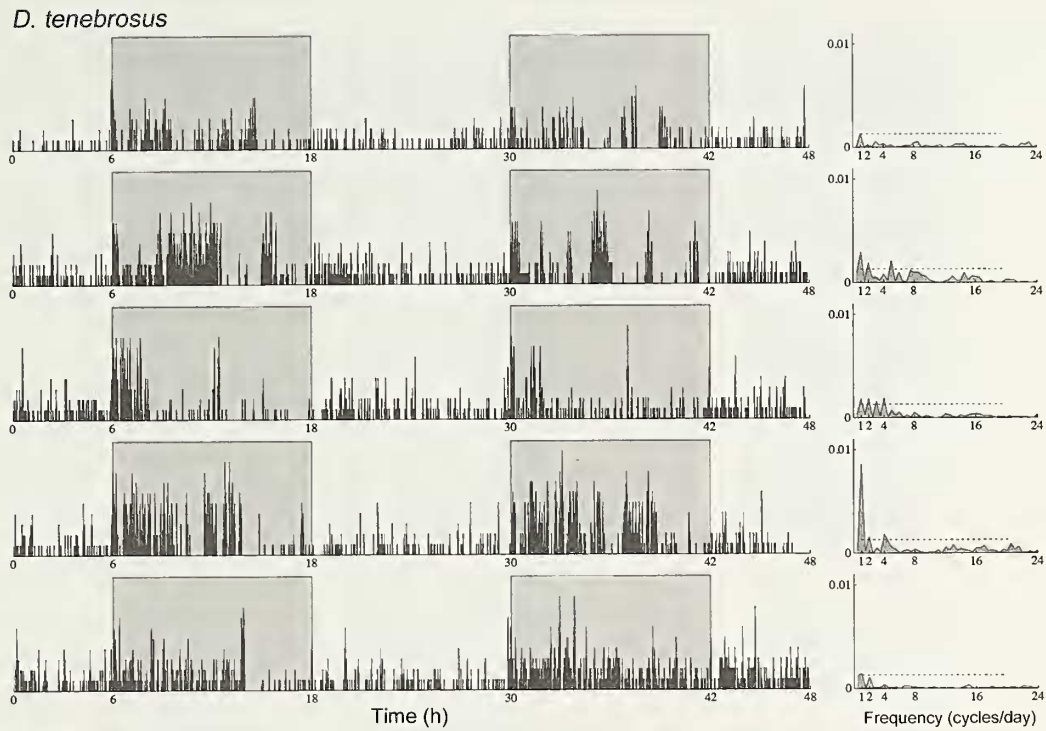


Figure 5.—*D. tenebrosus* (Pisauridae) short-term activity (left) and power spectra (right). The first two and the last two of these spiders were demonstrably nocturnal in their activity, but the middle spider's activity was approximately balanced between light and dark periods (Table 1). The power spectra show that all five of these spiders' activity patterns were periodic at about one cycle per day and all but the first and last were periodic at higher frequencies as well.

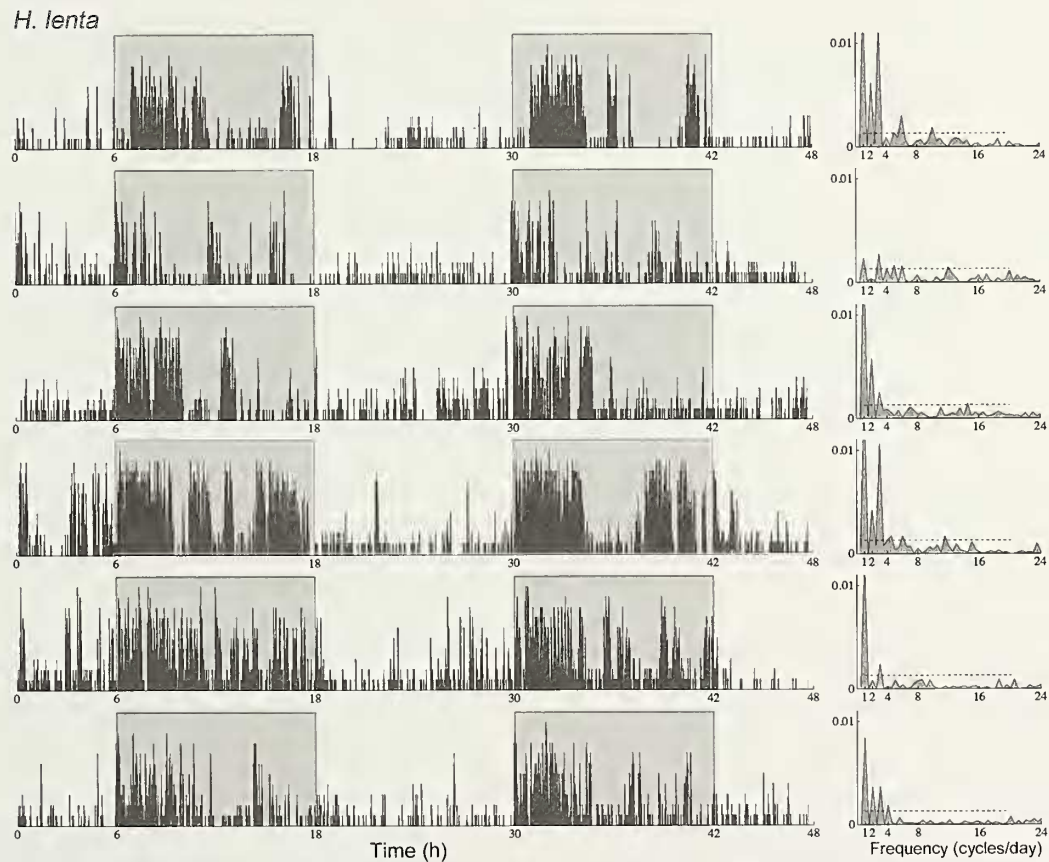


Figure 6.—*H. lenta* (Lycosidae) short-term activity (left) and power spectra (right). All of these spiders were strongly nocturnal in their activity (Table 2), and five of the six had power spectra showing highly significant periodicities at about one cycle per day. All of the spiders also had activity patterns that were periodic at higher frequencies as assessed with the discrete Fourier transform.

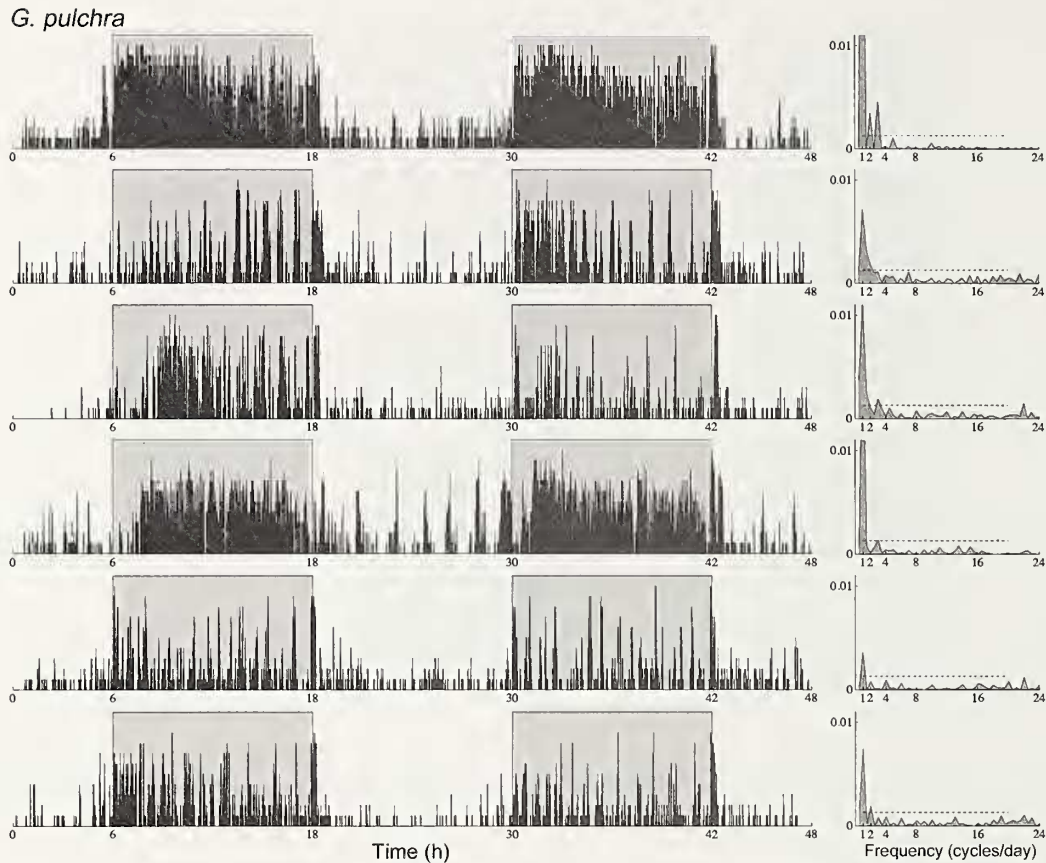


Figure 7.—*G. pulchra* (Lycosidae) short-term activity (left) and power spectra (right). All of these spiders were strongly nocturnal in their activity (Table 2), and all had significant periodicities at about one cycle per day. Five of the spiders also had activity patterns that were periodic at higher frequencies as assessed with the discrete Fourier transform. The first of these spiders appears to have been nearly continuously active during its periods of darkness, but this impression ignores the fact that only a few of the activity data reached the highest value of 1.0 which would signify that all of its ten component intervals contained activity.

We pooled the activity scores of the three pisaurid species and compared those to the pooled scores of the five lycosid species. The pisaurids' mean (\pm SE) activity level (801.7 ± 69.7 , $n = 15$) was less than half that of the lycosids (1750 ± 237.6 , $n = 31$) and the difference was significant (two-tailed $t_{44} = 2.733$, $P = 0.009$); the two groups' variances also differed significantly (Pisauridae \ll Lycosidae: $F_{14,30} = 24.00$, $P < 0.0001$).

Nocturnal vs. diurnal.—All three species of fishing spiders (Table 1; Figs. 3–5) and four of the five species of wolf spiders (Table 2; Figs. 6–9) were strongly nocturnal in their activity. Of all of the species we tested, only one, the wolf spider *Piratula minuta* (Table 2; Fig. 10), was diurnal. The metric supporting these statements was $(L-D)/(L+D)$, which could vary from -1 (fully nocturnal) to $+1$ (fully diurnal) (Tables 1, 2). The $(L-D)/(L+D)$ ratios did not differ among the fishing spider species (Table 1), nor did they differ significantly among the four nocturnal wolf spider species (Table 2), but the pooled nocturnal lycosids were more strongly nocturnal than the pooled pisaurids (two-tailed $t_{38} = 2.643$, $P = 0.0119$).

None of the individual spiders that showed statistically significant nocturnal or diurnal activity (31 of 31 lycosids, 14 of 15 pisaurids) was purely nocturnal or diurnal, an assertion supported both by the $(L-D)/(L+D)$ ratios (Tables 1, 2) and by visual inspection of the activity records (Figs. 3–10). That

is, in all individual cases except for the single ambivalent *D. tenebrosus* (Pisauridae), statistically strong nocturnality was accompanied by substantial activity during the light hours, and statistically strong diurnality (in all individual *P. minuta* and one individual *V. avara*) was accompanied by substantial activity during the dark hours.

Activity profiles.—We analyzed the activity profiles of the pisaurids (Fig. 11a) and lycosids (Fig. 11b) using these metrics: activity level surrounding dark onset, activity level surrounding light onset, and activity level trend through the dark period (as shown in Fig. 12). The fishing spiders and wolf spiders differed both with respect to within-family variability and with respect to overall levels of activity (above). The three pisaurids, all in the genus *Dolomedes*, had similarly shaped profiles (Fig. 11a) among which there were no significant differences in activity levels surrounding either the onset of darkness or the onset of light (Table 3), and there was a declining amount of activity during the dark hours that did not vary among the three species (Table 4).

The same consistencies were not present among the five species of wolf spiders (Fig. 11b). One difference, of course, was that *P. minuta* was predominantly diurnal in its activity, while the other four species were nocturnal. In addition, the lycosid data revealed that activity surrounding the onset of darkness and the onset of light varied significantly among the

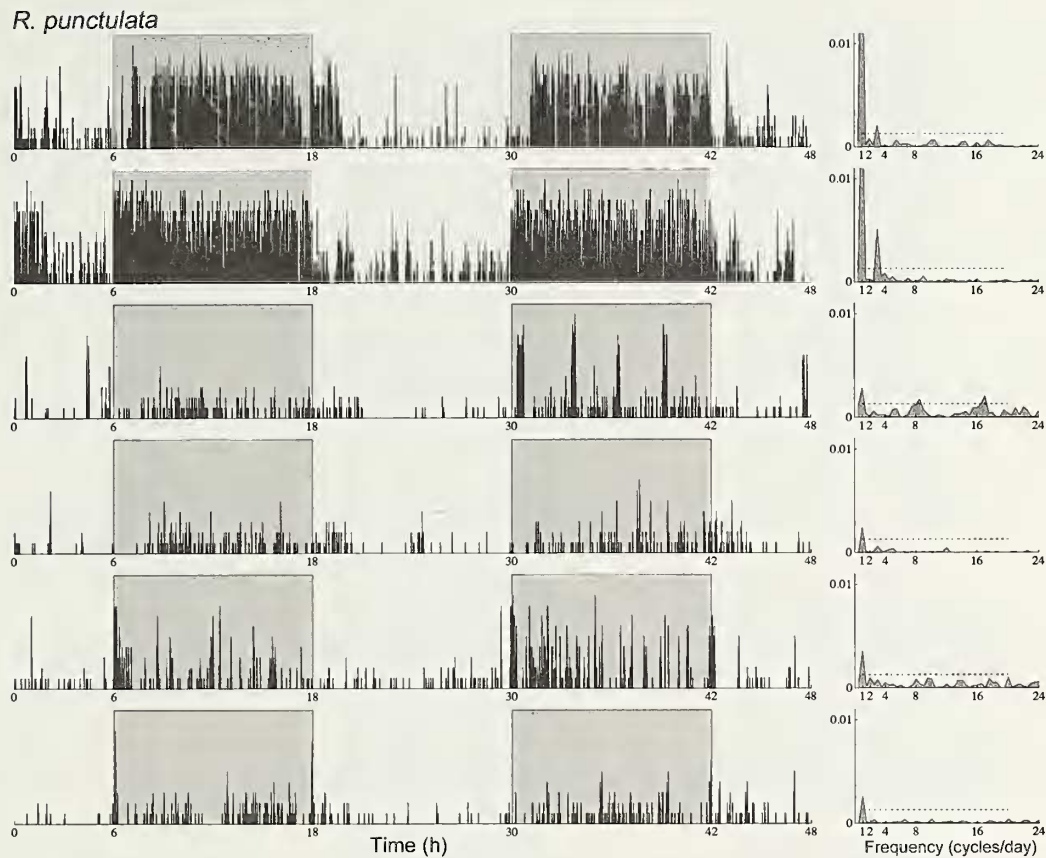


Figure 8.—*R. punctulata* (Lycosidae) short-term activity (left) and power spectra (right). All of these spiders were strongly nocturnal in their activity (Table 2), and all had significant periodicities at about one cycle per day. The first three spiders also had activity patterns that were periodic at higher frequencies as assessed with the discrete Fourier transform. The second spider appears to have been nearly continuously active during its periods of darkness, but this impression ignores the fact that only a few of the activity data reached the highest value of 1.0 which would signify that all of its ten component intervals contained activity.

species; *V. avara*'s conspicuous burst of activity immediately following the onset of darkness (Fig. 11b) was primarily responsible for the very low likelihood that the onset-of-darkness differences were due to chance alone (Table 3). Multiple linear regression showed that, during darkness, the activity of four of the wolf spiders (three that were nocturnal as well as the diurnal *P. minuta*) declined significantly while one (*R. punctulata*) had activity that did not change in intensity over the same period (Table 4). Although several of the lycosids displayed activity peaks early in the dark phase or early in the light phase (Fig. 11), none of them had the U-shaped profiles characteristic of crepuscular behavior in which most of the activity is expected to be concentrated at dusk and dawn (e.g., Nishimura et al. 2005). It is possible that some activity was suppressed by the abrupt light-to-dark and dark-to-light changes that we used during our trials, but the data in Figs. 3–10 do not show evidence of lasting suppression (as opposed to brief, transient suppression).

Periodicity of activity.—The power spectra (Figs. 3–10), revealing how much of the variability in a spider's activity was demonstrably periodic, showed that most spiders had significantly periodic activity at 1 cycle per 24 h and also at higher frequencies (frequency > 1/day, ultradian periodicities). Note that we cannot interpret significant periodicity at 1/day as evidence of an underlying physiological circadian rhythm. This is because, under a 12:12 LD light regime, that same

periodicity would emerge if activity during light hours were merely suppressed (in a nocturnal spider) or stimulated (in a diurnal spider). This is why, in general, the detection and measurement of endogenous circadian rhythms is carried out in constant darkness (Aschoff 1960).

The ultradian periodicities were conspicuously variable among species (Fig. 13), and were especially so among the lycosids. There, *V. avara* had by far the greatest number of significant periodicities at frequencies > 1/day and *P. minuta*, at zero, had the fewest. Overall, the fishing spiders were less likely to have higher frequency periodicities than were the wolf spiders, but most of that difference was attributable to the highly ultradian activity of one lycosid species, *V. avara* (Figs. 9, 13).

DISCUSSION

The subsets of fishing spiders (Pisauridae) and wolf spiders (Lycosidae) that we tested were interestingly different. The three congeneric species of fishing spiders were largely indistinguishable from each other, as might be expected because of their common ancestry, sharing overall activity levels, degrees of nocturnality, activity profile shapes, and having relatively few ultradian periodicities. In contrast, the five species of wolf spiders were not only different from each other but also, as a group, different from the fishing spiders.

The most conspicuous family-level differences were these: 1) the pisaurids were about half as active as the lycosids,

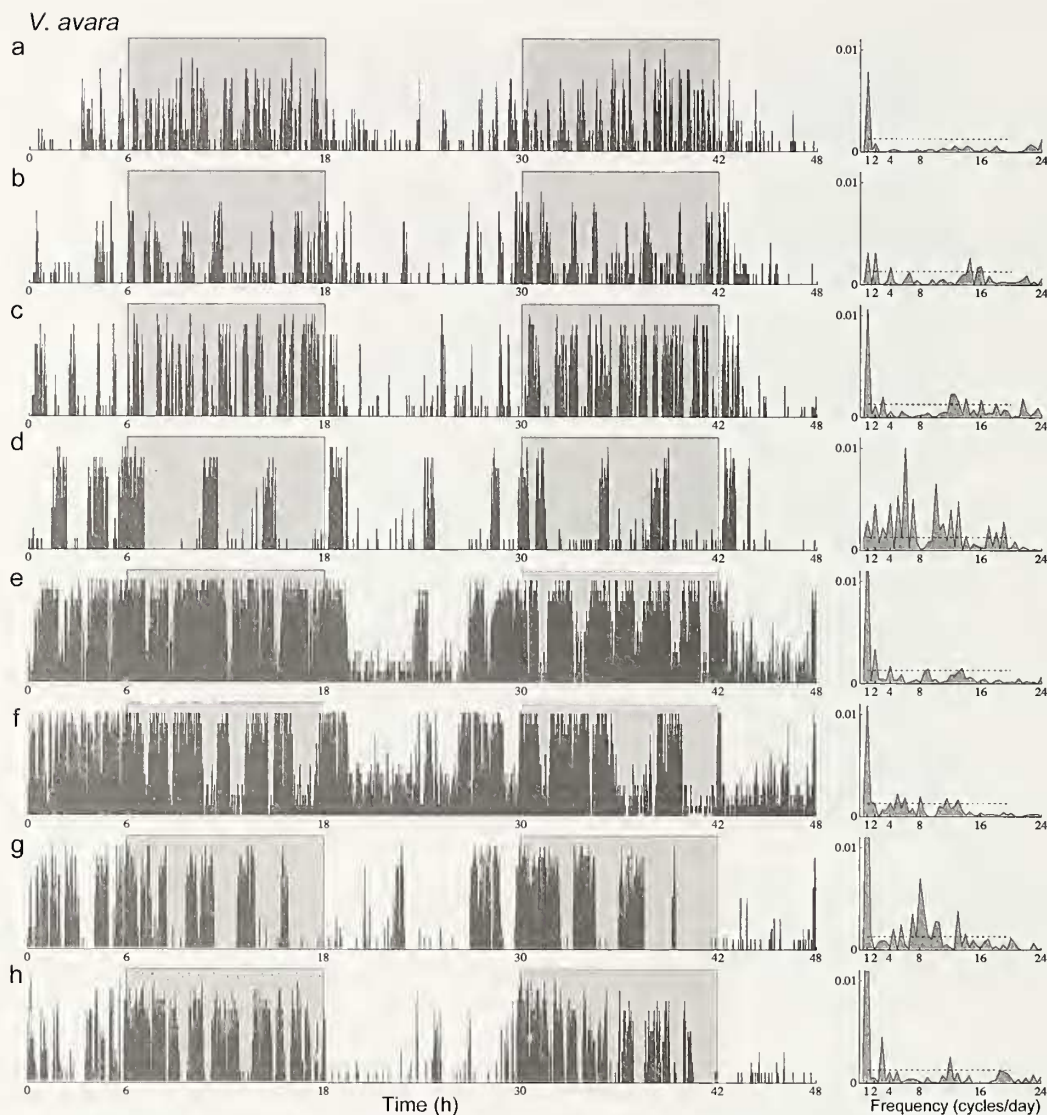


Figure 9.—*V. avara* (Lycosidae) short-term activity (left) and power spectra (right). Except for 9d, all of these spiders were strongly nocturnal in their activity (Table 2), and all including 9d had significant periodicities at about one cycle per day. All except 9a also had activity patterns that were periodic at higher frequencies than one/day. The spider represented by 9d was significantly diurnal (Table 2).

answering the first of our core questions; 2) the pisaurids were less strongly nocturnal than were the nocturnal lycosids (i.e., excluding the diurnal species, *Piratula minuta*), partially answering the second of our core questions; and 3) there were fewer ultradian periodicities in the activity patterns of the pisaurids than there were in the activity patterns of the lycosids, completing another part of the answer to the second of our core questions. Because these family-level differences were potentially biased by our use of three congeneric species in Pisauridae, we plan a broader sampling of that family in the future.

Spectrum of nocturnality.—Perusal of the activity records of the 46 spiders in this study (Figs. 3–10) shows that none of the individuals was purely nocturnal or purely diurnal. Each nocturnal spider had measurable activity during multiple time intervals while under light, and each diurnal spider was active during multiple time intervals while in the dark. The variability of $(L-D)/(L+D)$ was roughly continuous over most of its measured -0.71 to -0.10 range of nocturnality

(Tables 1, 2). (The same is likely to be true of diurnality, but we have too few data from this study to make that case.)

The spectrum of $(L-D)/(L+D)$ provides the answer to the third of our core questions: our measures indicate that none of the eight species tested was strictly nocturnal or diurnal. The five individuals with the lowest $(L-D)/(L+D)$ ratios came closest to being purely nocturnal, with 81.5–85.2% of their activity bouts occurring during darkness. Surprisingly, these five individuals represent five different species and two families, another indicator that the data in this study revealed substantial individual variation that may be found, upon further study, to be attributable to variables we did not control (e.g., genotype, time since last feeding, mating status).

The continuity of variability (Tables 1, 2) suggests that nocturnality in spiders may be a mutable characteristic, perhaps sensitive to the individual's history and current physiological state (proximate influences), or perhaps varying at the level of population genetics, possibly both. Whatever its underlying cause, and presuming that it is not an artifact of

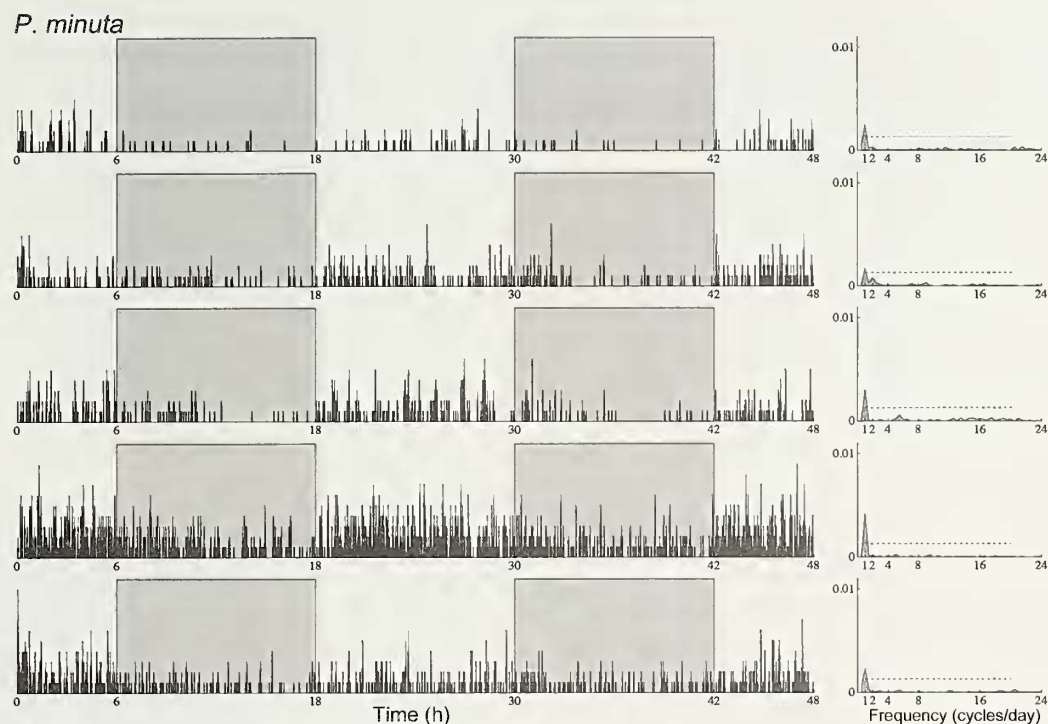


Figure 10.—*P. minuta* (Lycosidae) short-term activity (left) and power spectra (right). All of these spiders were strongly diurnal in their activity (Table 2), and all had significant periodicities at about one cycle per day. None had activity patterns that were periodic at higher frequencies than 1/day as assessed with the discrete Fourier transform.

the austere conditions of our test chambers (see “Quality of data” below), the variability should influence the way community ecologists think about syntopic spiders and the degree to which temporal partitioning can play a role in niche differentiation (e.g., Herberstein & Elgar 1994; Carrel 2003; Nieto-Castañeda & Jiménez-Jiménez 2009; Lapinski & Tschapka in press).

Bouts.—Our DFT analyses of the activity data (Figs. 3–10) indicate that, apart from the nearly ubiquitous presence of periodicity at 1 cycle/day, all of the spiders, except for one (*Piratula minuta*), also had significantly periodic activity pulses at higher frequencies (Fig. 13). As noted in the results section, the diel cycle of activity need not imply an underlying

circadian rhythm, although such a physiological rhythm is likely to be present (Cloudsley-Thompson 1978, 2000; Suter 1993; Jones et al. 2011). But the higher frequency (ultradian) periodicities in most spiders’ activity cannot have been driven by the 12:12 light regime and, in the very simplified environment of the experimental chambers, they also cannot have been driven by external cues. These ultradian periodicities appear to be one way that the spiders organize their activity into bouts.

The complexity of the etiology of bouts renders their initiation and duration difficult to understand empirically (Sugihara et al. 2012). Both stochastically initiated bouts and bouts that are the result of the interplay of several components

Table 3.—Activity profiles at the times surrounding the onset of darkness and the onset of light. We compared activity profiles among the pisaurids and the lycosids (via ANOVA) and between the pooled pisaurids and lycosids (t-test). In the ANOVA analyses, Tukey’s Multiple Comparison Test revealed significant pairwise differences only between *V. avara* and the other lycosids in the onset-of-darkness data (vs. *H. lenta*, $P < 0.01$; vs. *G. pulchra*, $P < 0.05$; vs. *R. punctulata*, $P < 0.001$; vs. *P. minuta*, $P < 0.01$). In both t-tests between the two families, the lycosids had significantly higher activity than the pisaurids, a correlate of overall higher activity levels (also see Fig. 11).

	Onset of darkness (hours 5.5, 6.0, 6.5) activity \pm SE (N)	ANOVA or t-test parameters	Onset of light (hours 17.5, 18.0, 18.5) activity \pm SE (N)	ANOVA or t-test parameters
<i>D. triton</i>	0.418 \pm 0.118 (5)		0.193 \pm 0.038 (5)	
<i>D. vittatus</i>	0.350 \pm 0.085 (5)	$F_{2,12} = 3.554, P = 0.061$	0.330 \pm 0.057 (5)	$F_{2,12} = 2.373, P = 0.135$
<i>D. tenebrosus</i>	0.677 \pm 0.064 (5)		0.248 \pm 0.036 (5)	
<i>H. lenta</i>	0.731 \pm 0.129 (6)		0.629 \pm 0.152 (6)	
<i>G. pulchra</i>	0.880 \pm 0.161 (6)		1.510 \pm 0.205 (6)	
<i>R. punctulata</i>	0.433 \pm 0.144 (6)	$F_{4,26} = 7.873, P = 0.0003$	0.839 \pm 0.344 (6)	$F_{4,26} = 2.893, P = 0.042$
<i>V. avara</i>	2.210 \pm 0.435 (8)		1.335 \pm 0.402 (8)	
<i>P. minuta</i>	0.437 \pm 0.145 (5)		0.207 \pm 0.069 (5)	
Pooled pisaurids	0.481 \pm 0.062 (15)		0.257 \pm 0.028 (15)	
Pooled lycosids	1.038 \pm 0.175 (31)	$t_{44} = 3.128, P = 0.0027$	0.954 \pm 0.151 (31)	$t_{44} = 2.168, P = 0.0356$

Table 4.—*Activity profiles* during darkness, excluding the onset of darkness and the onset of light (both covered in Table 3). We used multiple linear regression to quantify changes in activity over time (see Fig. 12), using the species-level mean profiles shown in Fig. 11. Except in the case of *R. punctulata*, the slopes were negative, with the passage of time accounting for 40–74% of the variance in activity. This was even true of *P. minuta*, the only species in this group that was demonstrably diurnal (Table 2).

	Slope of activity (hours 7–17) activity per hour	r^2	$F_{1,19}$	P	Slopes different?
<i>D. triton</i>	-0.0156	0.647	34.81	< 0.0001	$F_{2,57} = 1.64, P = 0.203$
<i>D. vittatus</i>	-0.0136	0.561	24.32	< 0.0001	
<i>D. tenebrosus</i>	-0.0205	0.732	52.01	< 0.0001	
<i>H. lenta</i>	-0.0607	0.601	28.61	< 0.0001	$F_{4,95} = 9.64, P < 0.0001$
<i>G. pulchra</i>	-0.0262	0.555	23.72	0.0001	
<i>R. punctulata</i>	-0.0034	0.044	0.881	0.359	
<i>V. avara</i>	-0.0373	0.400	12.69	0.002	
<i>P. minuta</i>	-0.0078	0.738	53.44	< 0.0001	

of the internal state, may have contributed to much of the “noise” surrounding the more regular periodic bouts of activity detectable in our data. We are working to tease apart the temporal organization of these data, but apart from the DFT analysis, those analyses (especially Markov chain and fractal analyses; Asher et al. 2009) are beyond the scope of this paper.

Intraspecific variation.—Several levels of intraspecific variation are evident in the data shown in Figs. 3–10. In the wolf spider *V. avara*, for example, the individuals were conspicuously variable in their quantities of activity (e.g., Figs. 9d vs. 9e), in the degree of nocturnality (the spider represented by Fig. 9d was significantly diurnal, the rest were nocturnal; see also Table 2), and in the presence of ultradian periodicities (e.g., Figs. 9a vs. 9b; see also Fig. 13). Even among the fishing spiders, in which we detected few species-level differences (Tables 1, 3, 4; Figs. 3–5, 11), individual-level differences were impressive. In *D. triton*, for example, the most nocturnal and the least nocturnal had $(L-D)/(L+D)$ ratios that differed by a factor of 6.9 (Table 1), and one of the five spiders showed no significant periodicity, even at 1 cycle per day.

These individual differences suggest that activity, as we have broadly measured it in this study, is neither strictly controlled by a light-dark cycle nor strongly patterned by an endogenous circadian rhythm or by immutable phylogenetic constraints. That is not to say that endogenous rhythms and phylogenetic constraints are lacking, but rather to note that other influences such as developmental history, recent experience, feeding history, and perhaps genetic variability within each species, are strong enough to override the influences not only of one nearly ubiquitous environmental variable, light, but also of the circadian rhythm and other innate patterning parameters.

Finally, we have some indication from intraspecific variability that transitioning from nocturnal to diurnal or vice versa may require only a gentle environmental or evolutionary nudge. First, the inter-individual variability in $(L-D)/(L+D)$ ratios (Tables 1 & 2) shows that, far from being a two-state discrete system, nocturnality and diurnality exist on a continuum; individuals and species near zero (50:50) would require only a small shift to cross from one nominal state to the other. And second, at least in the lycosid *V. avara*, where nocturnality appears to be the norm, an individual (Table 2, Fig. 9d) can be demonstrably diurnal. On the evolutionary side, if more were known about the phylogeny of the genera within the Lycosidae (Dondale 2005) it might be possible to

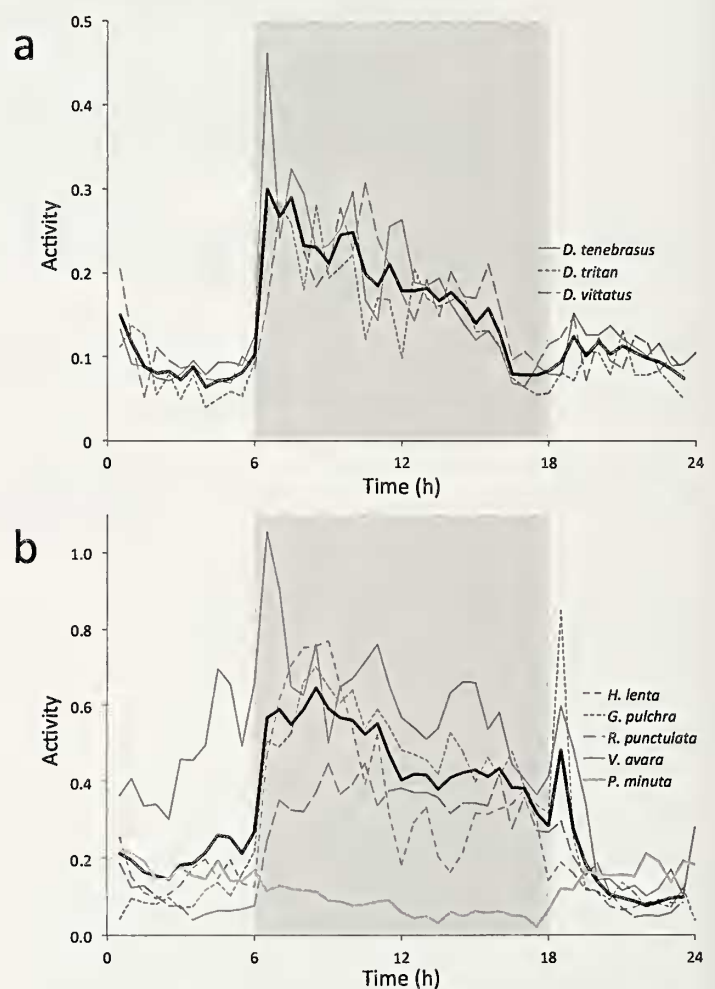


Figure 11.—*Activity profiles* of the pisaurids (a) and lycosids (b). The three species of pisaurids (a) are all in the genus *Dolomedes* and, perhaps because of that shared lineage, their *activity profiles* (gray lines) are similar and thus well represented by the average for the three species (black line). The five species of lycosids (b) represent five different genera (gray lines), four of which are nocturnal and one of them, *P. minuta*, is diurnal (Fig. 10, Table 2). Overall activity of the lycosids was higher than that of the pisaurids (note scale differences, and see Table 3). The data for *P. minuta* were excluded when we calculated the average (black line) for the lycosids. The shaded area on each graph indicates darkness in the experimental chamber.

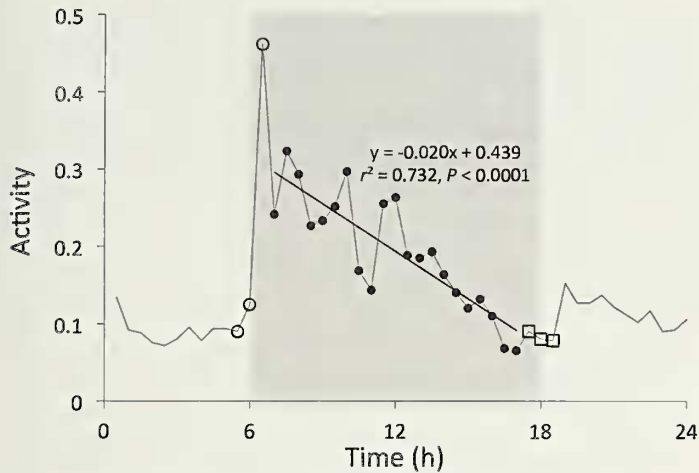


Figure 12.—Analysis of the activity profiles (Fig. 11) consisted of three parts, here shown for the activity profile of *D. tenebrosus*. Data surrounding the onset of darkness (open circles) were summed for each individual's profile, then compared via ANOVA across all species in the same family. Data surrounding the onset of light (open squares) were treated in the same way. The intervening data (filled circles) for each species were subjected to multiple regression analysis, a procedure that allowed us to determine both whether the slope of each species was $\neq 0$ and whether there were differences among the slopes of the species that could not be attributed to chance. The results of these analyses are reported in Tables 3 & 4.

infer whether, for example, the diurnal *P. minuta* (Fig. 10) is a recent and unusual convert to diurnality or is one species in a clade of other species and genera that are characteristically diurnal.

Quality of data.—We have two concerns about our data and analyses. First, we collected our data from individual captive spiders, each enclosed in an almost featureless chamber (see Methods). In vertebrates, the absence of environmental complexity while in captivity often leads to the expression of anomalous, sometimes repetitive behaviors (reviewed by Lewis et al. 2007), and there is no a priori reason to believe that arthropods are immune to such effects. Thus we recognize that some of the activity we detected and analyzed might not have been expressed by the spiders if they had been tested under more naturally complex conditions, and that our isolation of the spiders for testing may have led to similar artifacts. On the other hand, the activity captured by our monitoring apparatus did reveal patterns and differences that cannot have been caused by external influences and that might well have been obscured if we had collected our data under more natural conditions (dawn-like and dusk-like light transitions; prey available; refugia available). Further, in part because of the simplicity of our apparatus, we could automate data collection to a degree that would not be possible (or tractable) via most direct observational methods in more naturalistic settings, thus justifying the trade-off in external validity.

Our second concern is that the data we used in our analyses were quite far removed from the actual spider behaviors that caused changes in light levels at the IR sensors. The behaviors were analog motions (e.g., leg movements during locomotion, pedipalp movements during grooming, body shifts during postural changes) that we measured digitally. In terms of behavioral specificity, those measures were far less meaningful than, for example, the measures of wheel-running activity by mice (Suter & Rawson 1968; Hut et al. 2011) that have

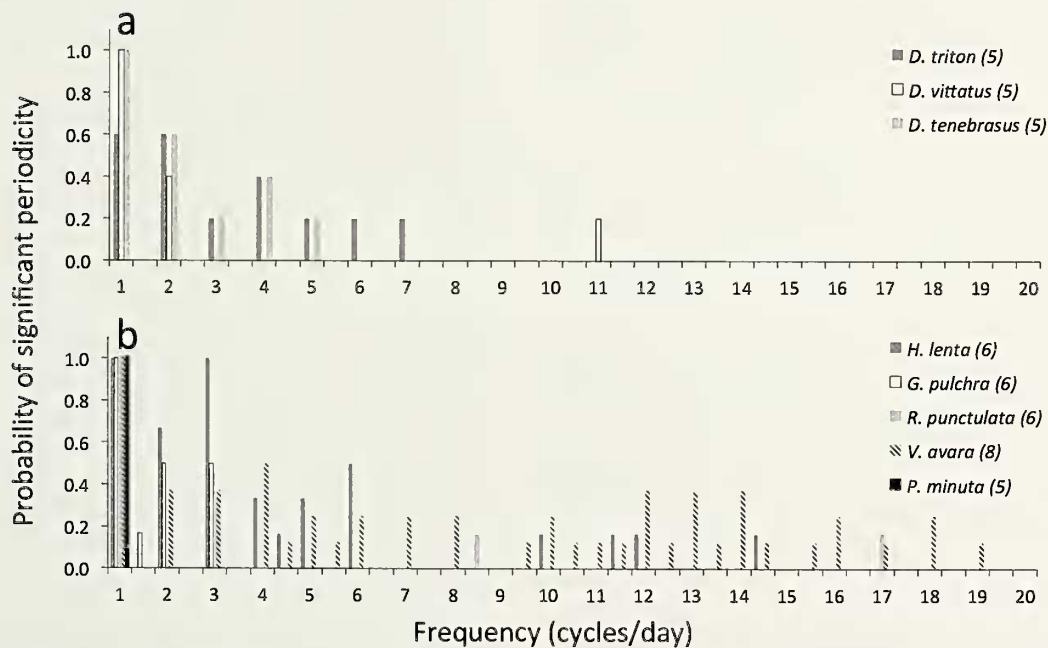


Figure 13.—Probability of a pisaurid (a) or a lycosid (b) evincing significant ($P < 0.01$) periodicity of activity at the frequencies shown. Nearly all individual spiders had the expected significant periodicity at 1 cycle/day with the exception that, in *D. triton*, two of the spiders did not show a significant peak at that frequency, hence the probability score of 0.6 for that species (3/5 of *D. triton* had a significant peak at 1 cycle/day). Overall, the lycosids were more likely to have high-frequency periodicities in their activity than were the pisaurids ($\chi^2 = 7.19$, $P = 0.007$, corrected for continuity; 1.5–7.0 cycles/day vs. 7.5–20 cycles/day), and this difference was largely due to the large number of significant periodicities in the activity of *V. avara*. On the other hand, only one species (*P. minuta*) among all tested had no significant periodicity at frequencies greater than 1 cycle/day. The legend shows N for each species.

populated the vertebrate circadian rhythm literature, or the assays of araneid defensive behavior devised by Jones et al. (2011). On the other hand, our measures integrate all forms of activity that result in motion and so, in a way, have the same general applicability that physiological measurements of metabolic rate have—the particular actions are not captured but the picture over time retains meaning (Fig. 2).

Summary.—All of the fishing spiders (Pisauridae) were nocturnal in their activity, as were four of the five species of wolf spiders (Lycosidae). All of those nocturnal species and the single diurnal wolf spider species, *Piratula minuta*, varied substantially in the degree to which they were nocturnal or diurnal, but none could be described as crepuscular. The most nocturnal of the spiders still performed 14.8–18.5% of their activity under the bright lights of their “day.” The (L-D)/(L+D) ratio of activity, which was the basis of our measure of nocturnal or diurnal tendencies, varied smoothly between the most nocturnal individual (-0.71) and the most diurnal individual (+0.62).

The pisaurids were less active overall than were the lycosids, and they were less strongly nocturnal than were the nocturnal lycosids (excluding the diurnal *P. minuta*). The pisaurids also had simpler temporal patterning than the nocturnal lycosids (again excluding the diurnal *P. minuta*)—our DFT analyses showed that, although all seven of the nocturnal species displayed periodic activity at frequencies $> 1/\text{day}$, periodicities at frequencies between 8–20 cycles per day were rare among the fishing spiders and relatively common among the wolf spiders.

ACKNOWLEDGMENTS

We are grateful to Patrieia Miller and Gail Stratton for their help in collecting our lycosid specimens and to Trigg Anderson for his help in collecting the pisaurids. Two anonymous reviewers and Elizabeth Jakob made suggestions that substantially improved an earlier version of this paper. Our study was supported in part by Lynchburg College and its Claytor Nature Study Center.

LITERATURE CITED

- Anderson, J.F. 1970. Metabolic rates of spiders. *Comparative Biochemical Physiology* 33:51–72.
- Aschoff, J. 1960. Exogenous and endogenous components in circadian rhythms. *Cold Spring Harbor Symposia on Quantitative Biology* 25:11–28.
- Asher, L., L.M. Collins, A. Ortiz-Pelaez, J.A. Drewe, C.J. Nicol & D.U. Pfeiffer. 2009. Recent advances in the analysis of behavioural organization and interpretation as indicators of animal welfare. *Journal of the Royal Society: Interface* 6:1103–1119.
- Blest, A.D. & W.A. Day. 1978. The rhabdomere organization of some nocturnal pisaurid spiders in light and darkness. *Philosophical Transactions of the Royal Society of London. Series B* 283:1–23.
- Blackledge, T.A., J.A. Coddington & R.G. Gillespie. 2003. Are three-dimensional spider webs defensive adaptations? *Ecology Letters* 6:13–18.
- Blackledge, T.A. & J.W. Wenzel. 1999. Do stabilimenta in orb webs attract prey or defend spiders? *Behavioral Ecology* 10:372–376.
- Carrel, J.E. 2003. Ecology of two burrowing wolf spiders (Araneae, Lycosidae). *Journal of the Kansas Entomological Society* 76:16–30.
- Cloudsley-Thompson, J.L. 1978. Biological rhythms in Arachnida. *Bulletin of the British Arachnological Society* 4:184–191.
- Cloudsley-Thompson, J.L. 2000. Biological rhythms in Arachnida (excluding Acari). *Memorie della Societa Entomologica Italiana* 78:251–273.
- Dondale, C.D. 2005. Lycosidae. Pp. 164–170. *In Spiders of North America: an Identification Manual*. (D. Ubick, P. Paquin, P.E. Cushing & V. Roth, eds.). American Arachnological Society, Keene, New Hampshire.
- Eisner, T. & S. Nowicki. 1983. Spider web protection through visual advertisement: role of the stabilimentum. *Science* 219:185–187.
- Finch, O.D. 2005. The parasitoid complex and parasitoid-induced mortality of spiders (Araneae) in a Central European woodland. *Journal of Natural History* 39:2339–2354.
- Forrest, T.G. & R.B. Suter. 1994. The Discrete Fourier Transform (DFT) in behavioural analysis. *Journal of Theoretical Biology* 166:419–429.
- Greenstone, M.H. & A.F. Bennett. 1980. Foraging strategy and metabolic rate in spiders. *Ecology* 61:1255–1259.
- Herberstein, M.E. & M.A. Elgar. 1994. Foraging strategies of *Eriophora transmarina* and *Nephila plumipes* (Araneae: Araneoidae): Nocturnal and diurnal orb-weaving spiders. *Australian Journal of Ecology* 19:451–457.
- Humphreys, W.F. 1974. Behavioural thermoregulation in a wolf spider. *Nature* 251:502–503.
- Humphreys, W.F. 1975. The influence of burrowing and thermoregulatory behaviour on the water relations of *Geolycosa godeffroyi* (Araneae: Lycosidae), an Australian wolf spider. *Oecologia* 21:291–311.
- Hut, R.A., V. Pistorz, A.S. Boerema, A.M. Strijkstra & S. Daan. 2011. Working for food shifts nocturnal mouse activity into the day. *PLoS ONE* 6(3):e17527.
- Jones, T.C., T.S. Akoury, C.K. Hauser & D. Moore. 2011. Evidence of circadian rhythm in antipredator behaviour in the orb-weaving spider *Larinioides cornutus*. *Animal Behaviour* 82:549–555.
- Kovoor, J., A. Muñoz-Cuevas & J. Ortega-Escobar. 1995. Diel morphological changes in the photoreceptors of *Lycosa tarentula* (Araneae, Lycosidae). *Biological Rhythm Research* 26:272–291.
- Kovoor, J., J. Ortega-Escobar & A. Muñoz-Cuevas. 1999. Circadian structural changes in the retina of *Lycosa tarentula* (Araneae, Lycosidae). *Biological Rhythm Research* 30:407–423.
- Kronfeld-Schor, N. & T. Dayan. 2003. Partitioning of time as an ecological resource. *Annual Review of Ecology, Evolution and Systematics* 34:153–181.
- Lewis, M.H., Y. Tanimura, L.W. Lee & J.W. Bodfish. 2007. Animal models of restricted repetitive behavior in autism. *Behavioural Brain Research* 176:66–74.
- Lapinski, W. & M. Tschapka. 2013. Habitat use in an assemblage of Central American wandering spiders. *Journal of Arachnology* 41:151–159.
- McQueen, D.J. & B. Culik. 1981. Field and laboratory activity patterns in the burrowing wolf spider *Geolycosa domifex* (Hancock). *Canadian Journal of Zoology* 59:1263–1271.
- Nieto-Castañeda, I.G. & M.L. Jiménez-Jiménez. 2009. Possible niche differentiation of two desert wandering spiders of the genus *Syspira* (Araneae: Miturgidae). *Journal of Arachnology* 37:299–305.
- Nishimura, T., N. Numata & E. Yoshioka. 2005. Effect of temperature on circadian rhythm controlling the crepuscular activity of the burying beetle *Nicrophorus quadripunctatus* Kraatz (Coleoptera: Silphidae). *Entomological Science* 8:331–338.
- Nørgaard, T., J.R. Henschel & R. Wehner. 2006. The night-time temporal window of locomotor activity in the Namib Desert long-distance wandering spider, *Leucorchestris arenicola*. *Journal of Comparative Physiology A* 192:365–372.
- Rovner, J.S. 1987. Nests of terrestrial spiders maintain a physical gill: flooding and evolution of silk constructions. *Journal of Arachnology* 14:327–337.
- Schmitt, A., M. Schuster & F.B. Barth. 1990. Daily locomotor activity patterns in three species of *Cupiennius* (Araneae, Ctenidae): The males are the wandering spiders. *Journal of Arachnology* 18:249–255.
- Schmitz, A. 2004. Metabolic rates during rest and activity in differently tracheated spiders (Arachnida, Araneae): *Pardosa*

- lugubris* (Lycosidae) and *Marpissa muscosa* (Salticidae). *Journal of Comparative Physiology B* 174:519–526.
- Sugihara, G., R. May, H. Ye, C. Hsieh, E. Deyle, M. Fogarty & S. Munch. 2012. Directing causality in ecosystems. *Science* 338:496–500.
- Suter, R.B. & K.S. Rawson. 1968. Circadian activity rhythm of the deer mouse, *Peromyscus*: effect of deuterium oxide. *Science* 160: 1011–1014.
- Suter, R.B. 1993. Circadian rhythmicity and other patterns of spontaneous motor activity in bowl and doily spiders. *Journal of Arachnology* 21:6–22.
- Suter, R.B. & T.G. Forrest. 1994. Vigilance in the interpretation of spectral analyses. *Animal Behaviour* 48:223–225.

Manuscript received 12 September 2013, revised 5 February 2014.